DETERMINATION OF TOTAL AND INDIVIDUAL ANTHOCYANINS IN RASPBERRIES GROWN IN SOUTH SERBIA

Milena Ivanović[1], Aleksandra Pavlović[1], Milan Mitić[1], Emilija Pecev Marinković[1], Jovana Krstić[1], Jelena Mrmošanin[1]

Abstract: Raspberry fruits of four cultivars (Willamette, Meeker, Polana and Malling Promise) grown in South Serbia were analyzed for total and individual anthocyanins. High-performance liquid chromatography with diode array detection (HPLC-DAD) was used to study individual anthocyanins. The major anthocyanin was cyanidin-3-sophoroside, followed by cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, pelargonidin-3-sophoroside and cyanidin-3-rutinoside. The monomeric anthocyanin contents of the acidified 80% methanol extracts were determined using the pH-differential method. The highest total and individual anthocyanins contain raspberries cv. Willamette, followed by cvs. Meeker, Polana and Malling Promise.

Key words: raspberries, individual anthocyanins, total anthocyanins, HPLC-DAD

Introduction

In recent years several berries such as the strawberry, blueberry, cranberry, and raspberry have been studied for their beneficial effects on health. These health benefits include prevention of certain types of cancer, cardiovascular diseases, type II diabetes, obesity, neurodegenerative diseases associated with aging, and infections (Mullen et al., 2002a; Cates et al., 2007; Paredes-Lopez et al., 2010).

Raspberries are a common and important fruit in the diet due to their content of essential nutrients and beneficial phytochemicals. Also, these fruits are of economic importance and widely consumed fresh, frozen, or in processed forms such as jellies, jams, and juices. In addition to their attractive color and superior flavor, raspberries contain wide range of polyphenolic phytochemicals, primarily the anthocyanins and ellagitannins (Rao and Snyder, 2010). Anthocyanin content can vary greatly by variety. The relative composition was cyaniding-3-sophoroside > cyanidin-3-glucorutonoside > cyanidin-3-glucoside > cyanidin-3-rutinoside > all pelargonidin glucosides combined (Rao and Snyder, 2010).

Official statistics reported that the raspberry production in Serbia in the last decade amounts for some of 80 000 t to 90 000 t produce on 15171 ha in 2010. The most of the production of raspberry is consumed within the country and small percentage is exported, mostly in frozen form. A very small percentage is exported as fresh (Centre for Development of Jablanica and Pcinja region with EPICENTAR International, 2012). Arilje growing area is the main production center (Leposavić et al., 2004).

[1]University of Niš, Faculty of Sciences and Mathemarics, Departmen of Chemistry, Višegradska 33, Niš, Serbia (milenai.chem@gmail.com).

The aim of this this study was determination of total and individual anthocyanins content in four varieties of raspberry grown in south Serbia.

Material and methods

Raspberry fruits (*Rubus idaeus L.*) of four cultivars (Willamette, Meeker, Polana and Maling promise) were collected from a natural population in the vicinity of Leskovac. Samples were harvested during June−August 2015. The amount of each raspberry cultivar collected for the analysis was about 500 g. Before analysis, the samples were stored in a freezer at −18 °C.

Samples were prepared according to the slightly modified method proposed by Pavlović et al. (2013). Two grams of homogenized fruit was mixed with 10 mL of methanol containing 1% HCl on ultrasonic bath for 1 h at room temperature. The extract was filtered, and the clear supernatant was collected. The fractions from three times repeated extractions were collected and evaporated to dryness by rotary evaporation under reduced pressure at 40 °C. Ultrapure water was added to ca. 10 mL, and these solutions were used for further analysis.

In order to identification and determine the individual anthocyanins content Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD) was used. The column was thermostated at 25 °C. After injecting 5 μL of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6×150 mm column. The mobile phase consisted of aqueous 5% formic acid (eluent A) and 80% acetonitrile/5% formic acid (eluent B). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and finally for the last 5 min gradually decreases 80-0% B. Identifications of individual compounds were based on the retention times of the available standards and spectral data (de Ancos et al., 1999; Mullen et al., 2002b; Lopes-da-Silva et al., 2002). Triplicate measurements were taken and data were presented as mean ± standard deviation (SD).

The monomeric anthocyanin contents of the acidified 80% methanol extracts were determined using the pH-differential method (Guisti and Wrolstad, 2001). The total anthocyanin content was calculated as micrograms of cyanidin-3-glucoside equivalents/100 g fresh weight (mg cyn-3-glu/100 g fw), using an extinction coefficient of 26.900 L/mol/cm and molecular weight of 449.2 g/mol.

Methanol, acetonitrile and formic acid (HPLC grade) and hydrochloric acid of analytical grade were purchased from Merck® (KGaA, Darmstadt, Germany). All standards for HPLC determination were purchased from Sigma-Aldrich (Steineheim, Germany) and were of HPLC quality.

Results and discussion

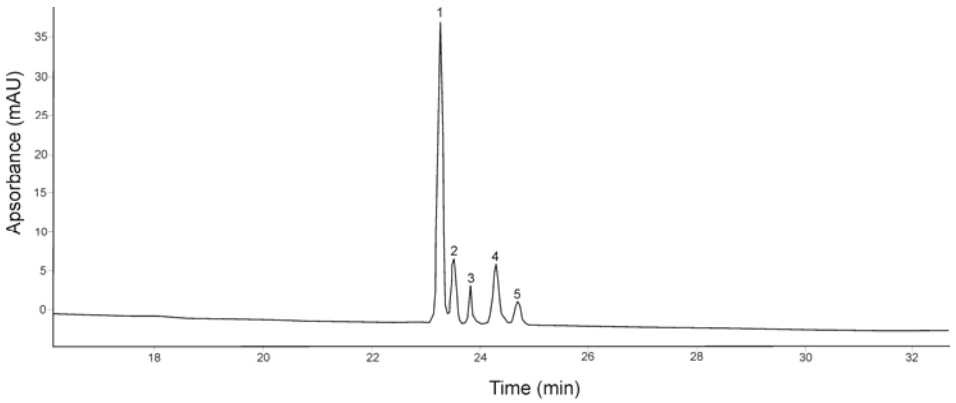
Anthocyanins are responsible for the black and red pigments in berries. For identification and quantification they were extracted with acidified 80% methanol. Results for total and individual anthocyanin contents are given in Table 1. The total

anthocyanin contents showed great variation in different cultivars. The total anthocyanins of the raspberry cultivars ranged from 16.2 mg cyn-3-glu/100 g (Malling Promise) to 47.6 mg cyn-3-glu/100 g (Willamette). These results are in agreement (Anttonen and Karjalainen, 2005; Kassim et al., 2009) or lower than results obtained by other researches (Pavlović et al.,2013; Miletić et al., 2012; Bobinaite et al., 2012; Miletić et al., 2015). This is in according to the fact that anthocyanin contents can vary by variety, geographical origin, climatic condition and handling procedures.

Tabela 1. Sadržaj ukupnih i individualnih antocijana u sortama maline
Table 1. Total and individual anthocyanin contents in raspberry cultivars

Cultivar	Cyanidin-3-sophoroside	Pelargonidin-3-sophoroside	Cyanidin-3-glucoside	Cyanidin-3-glucosylrutinoside	Cyanidin-3-rutinoside	Total anthocyanins *
	c_{sr}±SD (mg/100 g)					
Meekr	7.79±0.09	2.03±0.05	3.01±0.06	3.42±0.06	1.62±0.03	25.1±0.6
Polana	5.90±0.08	2.41±0.05	1.98±0.04	2.58±0.05	1.05±0.03	19.3±0.5
Willamette	20.4±0.5	2.85±0.06	5.78±0.08	6.18±0.09	2.47±0.05	47.6±0.8
Malling Promise	6.39±0.08	-	2.88±0.06	3.15±0.06	0.89±0.02	16.2±0.5

*Total anthocyanin contents are expressed as mg cyn-3-glu/100 g



Graph. 1. HPLC chromatogram of anthocyanins in raspberry sample. The labels refer to the following: (1) cyanidin-3-sophoroside; (2) cyanidin-3-glucosylrutinoside; (3) pelargonidin-3-sophoroside; (4) cyanidin-3-glucoside; and (5) cyanidin-3-rutinoside
Graf. 1. HPLC hromatogram antocijana u uzorku maline. (1) cianidin-3-soforozid; (2) cianidin-3-glukozilrutinozid; (3) pelargonidin-3-soforozid; (4) cianidin-3-glukozid i (5) cianidin-3-rutinozid

Results obtained for individual anthocyanins showed that the cyanidin-3-sophoroside was the predominant anthocyanin in all cultivars, followed by cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, pelargonidin-3-sophoroside and cyanidin-3-

rutinoside (Table 1, Graph 1). The contents and composition of anthocyanins in rasberry cultivars in the present study were comparable with those reported in the review work by Rao and Snyder (2010). The relative composition was cyanidin-3-sophoroside > cyanidin-3-glucosylrutinoside > cyanidin-3-glucoside > cyanidin-3-rutinoside > all pelargonidin glucosides combined. Also, cyanidin-3-sophoroside is known to be typical for European cultivars (de Ancos et al., 1999; Rao and Snyder, 2010).

Conclusion

The identification and quantification of individual anthocyanins revealed the similarities and variation in the composition and content of four raspberry cultivars. Cyanidin-3-sophoroside was the predominant anthocyanin in all of cultivars studied. Raspberries cv. Willamette contain the highest total and individual anthocyanins, followed by cvs. Meeker, Polana and Malling Promise.

Acknowledgment

This research was supported by grant numbers 172047 and 41018 from the Serbian Ministry of Education, Science and Technological Development. The authors are grateful for the financial support provided by this Ministry.

References

Anttonen, M. J., Karjalainen, R. O. (2005). Environmental and genetic variation of phenolic compounds in red raspberry. Journal of Food Composition and Analysis. 18 (8): 759-769.

Bobinaitė, R., Viņkelis, P., Venskutonis, P. R. (2012). Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars. Food Chemistry. 132 (3): 1495-1501.

Cates, E.M., Popa, G., Gill, C.I., McCann, M.J., Mc Dougall, G.J., Stewart, D. and Rowland, I. (2007). Colonavailable raspberry polyphenols exhibit anticancer effects on in vitro models of colon cancer. Journal of Carcinogenesis 6 (4): 4-15.

Centre for Development of Jablanica and Pcinja region with EPICENTAR International. (2012). Value chain analysis in southern Serbia. Available: http://www.fb.org.rs/en/upload/content/docs/VALUE_CHAIN_ANALYSIS_in_SOUTHERN_SERBIA.pdf

de Ancos, B., Gonzalez, E.M., Cano, M.P. (1999). Differentiation of raspberry varieties according to anthocyanin composition. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 208 (1): 33-38.

Guisti, M.M., Wrolstad, R.E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. Current Protocols in Food Analytical Chemistry. F1.2.1-F1.2.13.

Kassim, A., Poette, J., Paterson, A., Zait, D., McCallum, S., Woodhead, M., Smith, K., Hackett, C., Graham, J. (2009). Environmental and seasonal influences on red

raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL). Molecular Nutrition & Food Research. 53 (5): 625–634.

Leposavić, A., Milenković, S., Cerović, R. (2004). Raspberry production in the hilly mountainous region of Serbia. Journal of Mountain Agriculture on the Balkans, Troyan. 7 (3): 317-332.

Lopes-da-Silva, F., de Pascual-Teresa, S., Rivas-Gonzalo, J., Santos-Buelga, C. (2002). Identification of anthocyanin pigments in strawberry (cv. *Camarosa*) by LC using DAD and ESI-MS detection. European Food Research and Technology. 214 (3): 248-253.

Miletić, N., Leposavić, A., Popović, B., Mitrović, O. and Kandić, M. (2015). Chemical and antioxidant properties of fully matured raspberry fruits (*Rubus Idaeus L.*) picked in different moments of harvesting season. Acta Horticulturae. 1099: 211-218.

Miletić, N.M., Leposavić, A.P., Popović, B.T., Mitrović, O.V., Kandić, M.R. (2012). Contents of main phenolics and antioxidative capacity in frozen raspberry fruits (Rubus Idaeus L.) from Arilje growing area. 6th Central European Congress on Food, CEFood2012. Proceedings, 166-171, 23-26 May, Novi Sad, Serbia

Mullen, W., McGinn, J., Lean, M.E.J., MacLean, M.R., Gardner, P., Duthie, G.G., Zokota, T. and Croyier, A. (2002a). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. Journal of Agricultural and Food Chemistry. 50 (18), 5191-5196.

Mullen, W., Lean, M.E., Crozier, A. (2002b). Rapid characterization of anthocyanins in red raspberry fruit by high-performance liquid chromatography coupled to single quadrupole mass spectrometry. Journal of Chromatography A. 966 (1-2): 63-70.

Paredes-Lopez, O., Cervantes-Ceja, M.L., Vigna-Perez, M. and Hernandez-Perez, T. (2010). Berries: Improving human health and healthy aging, and promoting quality life – A review. Plant Foods for Human Nutrition. 65 (3): 299-308.

Pavlović, A.V., Dabić, D.Č., Momirović, N.M., Dojčinović, B.P., Milojković-Opsenica, D.M., Tešić, Ž.Lj., Natić, M.M. (2013). Chemical Composition of two different extracts of berries harvested in Serbia. Journal of Agricultural and Food Chemistry. 61 (17): 4188-4194.

Rao, A.V., Snyder, D.M. (2010). Raspberries in human health: A Review. Journal of Agricultural and Food Chemistry. 58 (7): 3871-3883.